Probiotics and prebiotics in intestinal health and disease: from biology to the clinic

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Accepted Version

Probiotics and prebiotics in intestinal health and disease: from biology to the clinic

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Abstract

Probiotics and prebiotics are microbiota management tools for improved host health. They target gastrointestinal effects via the gut, although direct application to other sites such as the oral cavity, vaginal tract and skin are being explored. Here, we describe gut-derived effects in humans. In the past decade, research on the gut microbiome has rapidly accumulated, accompanied by increased interest in probiotics and prebiotics as a means to modulate the gut microbiota. Given the importance of these approaches for public health, it is timely to reiterate factual and supporting information on their clinical application and use. In this Review, we discuss scientific evidence on probiotics and prebiotics, including mechanistic insights into health effects. Strains of *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces* have a long history of safe and effective use as probiotics, but *Roseburia* spp.*, Akkermansia* spp.*, Propionibacterium* spp. and *Faecalibacterium* spp. show promise. For prebiotics, glucans and fructans are well proven with evidence building on prebiotic effects of other substances (e.g. oligomers of mannose, glucose, xylose, pectin, starches, human milk; and polyphenols).

Key Points

- The human gut microbiota is integral to health and is associated with a variety of diseases
- Therapeutic and prophylactic effects of some probiotics and prebiotics for a variety of gut-related disorders might be, at least in part, mediated through modification of the microbiota and/or its function
- Probiotic microorganisms act via a variety of means, including modulation of immune function, production of organic acids and antimicrobial compounds, interaction with resident microbiota, interfacing with the host, improving gut barrier integrity and enzyme formation
- Prebiotics are substrates that are selectively utilized by host microorganisms conferring a health benefit; prebiotic effects include defence against pathogens, immune modulation, mineral absorption, bowel function, metabolic effects and satiety
- Use of some probiotics and prebiotics is justified by robust assessments of efficacy, but not all products have been validated; the goal is evidence-based use by healthcare professionals

[H1] Introduction

When the Argentinian government requested of the Food and Agriculture Organization of the United Nations that an expert panel be formed to evaluate the health and nutritional properties of probiotics in food in 2000, it precipitated the re-emergence of a concept long part of human history. International recognition of the concept of probiotics, and coalescence around a definition of probiotic offered by this expert consultation,[1] established an important consensus foundation.

The definition of probiotic decided by the consultation retained the essence of historical definitions offered over previous decades. It was intentionally broad, to encompass a wide variety of microorganisms, hosts, benefits, target sites and product types. It has stood the test of time and was reaffirmed, but grammatically corrected, in 2014 to the consensus definition of probiotics, which is: “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”.[2]

Studies abound that describe how microbes are integrated into life processes and define ways that beneficial microorganisms—both commensal and externally applied—affect physiological homeostasis and host function.[3] On the horizon is the promise of newly constructed recombinant strains and promising novel microbial species, which await testing *in vivo*. However, as these advances develop, we should recognize actionable evidence that is currently available. As will be discussed, convincing evidence exists for some established probiotics, which should be incorporated into health management. This incorporation includes complementary use with pharmaceutical agents, foods and lifestyle. Education of consumers, practitioners and regulators will facilitate appropriate use and point out needs for further research, which will hopefully include exploration of how to reach the individuals at greatest need with affordable and reliable probiotic products.[4]

Prebiotics, first defined in 1995[5], have been used to manipulate microbes in the host to improve measurable health outcomes. An update to the prebiotic definition published in 2017 as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” was compelled by the need to clarify what did and did not constitute a prebiotic substance in the face of scientific advances.[6] The desire to optimize, for improved health, the microbial world associated with us has led to the development of compounds targeting an ever-expanding group of microorganisms and benefits that are derived through them. No longer are prebiotics seen simply as boosters of the growth of bifidobacteria and

lactobacilli, with recognition of their effects on system-wide metabolic and physiological readouts.[6] Although the intestine remains the gateway to most of these effects, it is not an exclusive one. The extent to which prebiotics can affect microbial communities of the urogenital tract, oral-nasal areas and skin is now the subject of intensive exploration.[7]

This Review describes the current understanding of probiotic and prebiotic mechanisms of action, provides important examples of clinical studies on probiotic and prebiotic applications, and discusses current knowledge on mechanisms at the heart of these effects.

[H1] Human gut microbiome

The human gut is predominantly inoculated at birth. Microbial diversity develops as feeding and dietary patterns mature. It resembles the 'adult-like' microbiota after 3-5 years.[8] Because of variations in pH, substrate concentration, Eh (redox potential, activity of electrons) and transit time, microbial numbers vary between different anatomical regions of the gut.[9] The stomach harbours fewer microbes than the small and large intestines.[10] Studies using metagenomic approaches have highlighted the complex inter-relationship between our resident intestinal microbiota and mammalian metabolism.[11] Through the process of fermentation, anaerobic gut bacteria metabolise substrates to form end products such as organic acids and gases.[12] The main precursors for fermentations are dietary carbohydrates, proteins and lipids, as well as indigenous secretions such as mucin. This anaerobic metabolism contributes positively towards host daily energy requirements and homeostasis in the gut.[13] Ideally, the human host lives in harmony with its complex gut microbiota in a state that promotes physiological resilience.[14] However, dysbiosis can result from challenges such as medications, infections, ageing, lifestyle, surgery and poor nutrition,[14,15]

In humans, a range of acute and chronic disorders can be a consequence of perturbation of gut microbial communities.[16-18] On a chronic basis, inflammatory bowel disease (IBD), obesity and irritable bowel syndrome (IBS) have all been linked to intestinal bacteria and their activities.[10] This aspect opens up the possibility of influencing the microbiota to reduce disease risk, fortify homeostasis and, in some cases, improve therapeutic status. Diet is a principal driver of gut fermentation and therefore can greatly influence functionality of the indigenous microbiota.[19] Prebiotics

are popular dietary approaches for modifying the gut microbiota to improve host health,[6] as they are affordable, effective, safe and accessible.

[H1] Probiotics

As the concept of probiotics evolved over the past decades, the assumption was that their effects would be mediated through direct interaction with commensal microbiota. Some early definitions stipulated that probiotics functioned "by contributing to (the host's) intestinal microbial balance"[20] or "by improving the properties of the indigenous microflora".[21] However, the current consensus definition of probiotics does not stipulate that probiotic effects are only microbiota-mediated, and indeed, other types of mechanisms are known. This idea that probiotics function in ways that might act beyond affecting the colonizing microbiota opens the door to a wider range of probiotic possibilities, encouraging innovation in the field.

Much of our knowledge on probiotic mechanisms is based on research using *in vitro*, animal, cell culture or *ex vivo* human models. Figure 1 compiles known mechanisms distributed among various probiotic strains. Not all mechanisms have been confirmed in humans nor do they exist in every probiotic strain. Although multiple mechanisms likely co-express in a single probiotic, the importance of any given mechanism will depend on many factors. For example, in an inflamed intestine, the ability to down-regulate inflammatory mediators and increase epithelial barrier function may be most important,[22,23] whereas the ability to increase short chain fatty acids and hydration in the colon may be more important to normalizing intestinal motility.[24]

Research elucidating mechanisms of probiotics often relies on *in vitro* or animal studies. Probiotics are not unique in this regard. Animal studies have not always translated to humans;[25] notable examples are probiotics for Crohn's disease and mental health function.[26,27] Furthermore, inherent difference among probiotic strains exists, for example in the findings that one probiotic (in this case in conjunction with a prebiotic) significantly prevented sepsis in infants,[28] whereas a different formulation failed to prevent necrotizing enterocolitis.[29]

The historic concept of 'colonization resistance,'[30] the situation whereby native gut microbiota occupy host tissues to exclude infection by potential pathogens (resident or invading), is another mechanism attributed to probiotics.[31] Expression of colonization resistance is likely a sum outcome of the functioning of many of these different mechanisms in concert. Indeed, many host factors may impact the ultimate

expression of health effects imparted by a probiotic, including properties of baseline microbiota. Although few data exist, one study tracking probiotic persistence in the gut was linked to properties of the baseline microbiota. Persistence of *B. longum* subsp. *longum* AH1206 in the human gut was predicted by low abundance in the host of *B. longum* and low microbial carbohydrate utilization genes.[32] No clinical endpoints were tracked in this study, but the property of long-term persistence may contribute to physiological benefits. However, results of many different clinical trials that do not include stratification of subjects by baseline microbiota suggest that probiotic function is not necessarily predicated on a specific microbiota baseline.[33,34] There may well be compositional patterns of microbiota that do not respond well to incoming probiotic strains, just as there are for certain drugs,[35] but such profiles have not yet been fully defined.

***[H3] Modulation of cell-mediated and humoral immune functions*.** Some probiotics have been shown to increase phagocytosis or natural killer cell activity and interact directly with dendritic cells (reviewed in[36]) Some also demonstrate the ability to upregulate antibody secretion translating into improved defences against pathogens and augmenting vaccine responses.[37-39] Probiotic strains can increase levels of anti-inflammatory cytokines such as tumour necrosis factor with implications for abating colon cancer and colitis.[10,36] As discussed later, cell-surface architecture, such as fimbriae, capsule and surface structures expressed by certain probiotics is a mechanistic driver for several of these activities.

[H3] Production of organic acids*.* Probiotic species belonging to the *Lactobacillus* and *Bifidobacterium* genera produce lactic and acetic acid as primary end-products of carbohydrate metabolism. These organic acids when produced *in situ* can lower luminal pH and discourage growth of pathogens as shown in various model systems.[40-42] *Lactobacillus* and *Bifidobacterium* do not produce butyrate but through cross-feeding other commensal microbiota (for example, *Faecalibacterium*), levels of butyrate and other short-chain fatty acids in the gut can increase, potentially influencing many aspects of physiology, including the cardiometabolic phenotype.[43] This phenotype can be derived from increased production of butyrate, correlating with improved insulin response, or abnormalities in propionate linked to type 2 diabetes.[44] Based upon analyses of weight, lifestyle, metabolic measurements and short chain fatty acid (SCFA) levels, the risk of subjects developing cardiometabolic diseases can be calculated.[45]

[H3] Interaction with gut microbiota. Probiotic strains can interact with the gut microbiota through competition for nutrients, antagonism, cross-feeding and support

of microbiota stability.[46] Many probiotic strains are antagonistic toward other microbes, in part due to saccharolytic metabolism, which produces organic acids, but also by production of bacteriocins.[47] These antimicrobial compounds can be active against pathogens at many sites including the human urinary tract and in the gut of humans or animals.[48,49] Bifidobacteria produce acetate and can cross-feed other members of the gut microbiota (reviewed in[50]). Strains *B. longum* AH1206 and *B. bifidum*-ATCC15696 have been shown to persist in the infant gut,[32,51] although in the latter case the concomitant decrease in pathogen abundance was not tested for a link to bacteriocin production. The ability of certain probiotic strains to improve eradication of *Helicobacter pylori* may involve some inhibition of the pathogen, but stronger evidence for probiotics in this context is for reducing side effects of antibiotics used in treatment.[52]

[H3] Probiotic–host interactions. Interactions of probiotic strains with host tissues are mediated by cell surface macromolecules, including proteins (surface layer associated proteins, mucin binding proteins, pili, and LPxGT-binding proteins) and non-protein components (lipoteichoic acid, peptidoglycan, exopolysaccharides).[53] These structures have been shown to affect binding to intestinal and vaginal cells, mucin, and immune or dendritic cells resulting in increased transit times and improved barrier integrity (reviewed in[53]). An example of the different surface structures can be seen in the genome comparison of *L. rhamnosus* GG that uses pili to interact with the intestine and *L. rhamnosus* GR-1 with a unique cluster of exopolysaccharides to aid in vaginal activity.[54]

[H3] Improvement of barrier function. Primarily through studies in cell lines, several probiotic *Lactobacillus* and *Bifidobacterium* strains have been shown to increase expression of tight junction proteins (reviewed in[55]). A study using human intestinal epithelial enteroids and colonoids showed that *L. rhamnosus* GG pre-treatment countered damage to tight junction zonula occludens 1 (ZO-1) and occludin (OCLN) caused by interferon-gamma.[56] Another way in which probiotic strains may improve barrier function is through upregulating expression of mucus secretion genes, thereby reducing pathogen binding to epithelial cells.[57,58]. Down-regulating inflammation is also regarded as a factor that improves barrier function.[53] Of note, although some probiotic strains have the capacity to improve barrier function, this does not always occur in every cohort for reasons not yet fully understood.[59]

[H3] Manufacture of small molecules with local and non-local effects*.* Small molecules produced by certain probiotic strains have been described with different

effects on the host and its microbiota.[58] Perhaps one of the more intriguing findings is the production of neurochemicals such as oxytocin, gamma-amino butyric acid, serotonin, tryptamine, norepinephrine, dopamine and acetylcholine (reviewed in[60, 61,62]) known to affect brain function. In a rat model of stress, *L. helveticus* NS8 feeding resulted in lower plasma corticosterone and adrenocorticotropic hormone levels and restored hippocampal serotonin and norepinephrine.[63]

[H3] Production of enzymes. Microbial enzymes such as β-galactosidase[64] and bile salt hydrolase,[65] which are produced and delivered by some probiotic strains, improve lactose digestion and blood lipid profiles in humans, respectively. In the case of *Streptococcus thermophilus* in yogurt, which facilitates lactose digestion, its predisposition to be permeabilized by bile when entering the small intestine promotes the delivery of microbial β-galactosidase to the small intestine to break down lactose into digestible glucose and galactose.[64] This results in clinical benefit to individuals who are lactose intolerant. Indeed, the European Food Safety Authority considered evidence of this effect sufficient to authorize a health claim for *S. thermophilus* and *L. bulgaricus* as components of yogurt to alleviate symptoms of lactose maldigestion.[66]

Admittedly, cause and effect evidence of mechanisms in human hosts remains to be gathered, but technological advancements in genome sequencing and microbiome analyses, and surgical advances that allow real-time sampling *in vivo*, should help acquire elucidating data over the next few years.

[H1] Prebiotics

If we are to understand how prebiotics work, and more importantly exploit them to manipulate the microbiota to propagate health, then we need to keep in mind that microbes live in complex functional ecosystems. Within these, bacteria have a multitude of roles, including the conversion of incoming dietary carbohydrates, proteins and some fats into metabolites that can have either positive or negative effects upon host health.[67,68,69,70]. Current prebiotics are predominantly carbohydrate-based, but other substances such as polyphenols and polyunsaturated fatty acids may exert prebiotic effects.[6] An example of polyphenols is water-insoluble cocoa fraction, shown in a gut model to significantly increase bifidobacteria, lactobacilli and butyrate production.[71]

Low molecular weight carbohydrates are very efficiently metabolised by microorganisms such as bifidobacteria, which possess a range of cell-associated and extracellular glycosidases and specific transport systems allowing them to rapidly

assimilate low molecular weight sugars.[72,73] Other microbes, such as members of the *Bacteroides* genus are adept at breaking down high molecular weight polysaccharides.[74,75] Some bacteria might be regarded as keystone, having the ability to initiate breakdown of particular substrates;[76] for example, *Ruminococcus* spp. can facilitate resistant starch degradation.[77] Liberated low molecular weight dextrins are then metabolized by the microbial community. The pathway from a polysaccharide to a SCFA is thus a complex and indirect network of metabolism. Acetate and lactate, the main metabolic end products of bifidobacteria and lactic acid bacteria, are utilised by other microorganisms to produce, for instance, propionate[78] and butyrate.[50,79] Likely ecological networks involved in the metabolism of carbohydrates have been elucidated,[74,80,81] although the extent to which they operate in the gut is not clear at the present time.

A further complication in studies of the ecosystem response to carbohydrates is that it is heavily influenced by the microorganisms that are already present. It has become clear that individual microbiomes that are *Prevotella*-dominant can ferment carbohydrates more rapidly than can *Bacteroides*-dominant microbiomes.[82] Furthermore, when these distinct faecal inocula, dominated by *Prevotella* or *Bacteroides*, were incubated with prebiotic fructo-oligosaccharides (FOS) or with two different arabinoxylans, the profile of SCFA produced was distinctly different and correlated with the microbiome.[83] Cultures using *Prevotella*-dominant inocula produced significantly higher ratios of propionate to acetate and butyrate than the *Bacteroides*-dominant microbiotas. A similar influence of starting microbiome composition on carbohydrate fermentation has been seen using isomalto-oligosaccharides as a carbon source in an *in vitro* batch fermentation model with human microbiota.[84]

Microbiome studies based on 16s rDNA sequencing have given rise to an increased awareness of the richness of the gut microbial ecosystem[85] and, in some cases, to associations between certain microorganisms or microbiome profiles and disease states. These include IBD,[86] type II diabetes mellitus,[87-89] IBS[90,91] and obesity.[92,93]. These profiles have frequently been termed “dysbioses”, although it is not currently possible to define such a state as ‘normobiosis’ or a ‘normal’ microbiota Such associations tend to be merely the starting point for investigation into the role of specific microorganisms in disease. Sequencing studies do not give us an understanding of the functional interactions between members of the gut microbiota and it is imperative that this functional ecology is studied in more detail. It is becoming clear that although there might be a huge diversity of individual taxa in the gut

microbiomes of individuals, there is a high level of functional redundancy, and specific ecological functions are provided by a range of bacteria across different individuals,[94,95].

Given that we have an imperfect understanding of the functional ecology of the gut microbiota, uncovering the mechanisms of action of prebiotics presents a challenge. Despite this issue, we can postulate probable mechanisms by which a prebiotic can lead to health benefits. These pathways are presented in Figure 2 and discussed herein. All of these postulated mechanisms have support from research carried out through *in vitro* or animal models, although in many cases, establishing that they actually occur within human gut microbiota is difficult.

[H3] Defence against pathogens. Although mechanistically challenging to establish in humans *in vivo*, pathogen defence can be investigated *in vitro* using model systems.[96,97]. As noted for probiotics, production of organic acids through prebiotic administration and propagation of beneficial bacteria will result in a reduction in luminal pH, inhibiting growth of pathogens. Establishment of a stable population of commensal microorganisms will reduce nutrient availability for invading microorganisms, inhibiting colonisation. In studies of elderly individuals, 10 weeks of daily galactooligosaccharide (GOS) intervention induced increases in immune function, notably enhanced phagocytic activity and activity of natural killer cells.[98,99].

[H3] Immune modulation*.* Although exact mechanisms are unclear, there is evidence that prebiotic intervention can reduce type 2 T helper responses and thus affect allergy. The most supportive data come from studies in infants. Galacto- and long chain fructo-oligosaccharides in infant formula administered in a double-blind, randomized, placebo-controlled trial of 259 infants, showed reduced incidence of atopic dermatitis, wheezing and urticaria to less than 50% of the incidence in non-prebiotic formula fed infants.[100,101] In a prospective, double blind, placebo-controlled fashion, not as yet replicated, healthy term infants at risk of atopy fed prebiotic-supplemented hypoallergenic formula for 6 months had a greater than five-fold reduction in prevalence of allergies five years after feeding.[102]

[H3] Increased mineral absorption. Most absorption of minerals takes place by active transport mechanisms in the small intestine,[103] Scavenging calcium could make a substantial positive contribution to health. As already discussed, fermentation of prebiotics leads to production of SCFA, which reduces luminal pH. This drop in pH can increase calcium solubility, thereby providing a greater driving force for passive uptake. A problem with proving this is that many calcium salts in supplements and

food have pH-dependent solubility and limited availability, and depending on the starting pH, solubility of calcium can actually increase with higher pH.[104]

Studies have shown that consumption by young adolescents of a mixture of FOS and inulin[105,106] or GOS[107] can result in marked increases in absorption and calcium mineralised into bone. Such early intervention may reduce incidence of osteoporosis in later in life. This hypothesis is supported by animal model data[108], but long-term studies in humans are lacking.

[H3] Improved bowel function. Improvements in bowel function have often been ascribed to simple faecal bulking by consumption of dietary fibre. However, animal studies have shown that SCFAs produced by fermentation of prebiotics can regulate gut hormones that in turn modulate the local motor responses of the gut[109,110]. The humectant water binding capacity of prebiotic carbohydrates also has the effect of softening stools, making passage easier.[111,112]

There are surprisingly few studies on the effect of prebiotics on bowel function, although they have consistently led to improvements in stool consistency and defecation frequency in randomized trials[113,114]

[H3] Metabolic effects. As discussed earlier, prebiotic intervention results in the elaboration of SCFAs that can act to improve barrier function in the gut and prebiotic intervention with GOS has shown improvements in barrier function *in vivo.*[115] Impaired barrier function can allow translocation of inflammatory mediators such as bacterial lipopolysaccharide (LPS) from the gut into systemic circulation, which has been termed metabolic endotoxaemia[116] and has been suggested to be a causative factor in diabetes and obesity according to evidence, albeit from studies in mice.[117, 118].

Metabolic effects of prebiotics have been subject to several meta-analyses[119,120,121,122] and although the results among studies vary, the general consensus is that prebiotic intervention has a positive effect on glucose homeostasis, inflammation and blood lipid profile in humans. Although interventions with GOS[123] and inulin[124] have shown improvements in inflammatory markers in individuals with obesity, these have been relatively short-term studies over a few months and the effect on metabolic health over a long period of consumption is yet to be established.

The hypothesis underlying much research on prebiotics and barrier function and inflammation is that fermentation products such as SCFA probably mediate the beneficial effects through mechanisms discussed above. However, it has been shown that, at least *in vitro*, GOS can directly stimulate the expression of tight junction proteins in intestinal epithelial cell lines and decrease trans-epithelial flux[125,126]. Given

that GOS is fermented in the gut, however, the extent to which such mechanisms act *in vivo* are unclear at present.

It is possible that the effect of inulin in improving glycaemic response could be due to direct inhibition of the intestinal isomaltase-sucrase enzyme complex, but so far the evidence is only from mouse studies[127]. Identification of direct mechanisms from metabolic mechanisms in humans is, however, extremely difficult.

[H3] Effect on satiety. SCFAs produced by fermentation in the gut can interact with specific fatty acid receptors, FFAR2 and FFAR3 and regulate lipolysis and release of the incretin glucagon-like peptide-1.[128,129] These receptors are found on many tissues and could be a key mechanistic link between prebiotic fermentation and systemic health benefits. SCFAs can regulate appetite via several mechanisms,[130] with studies showing that the interaction between SCFA and colonic L-cells results in production of anorexigenic hormones such as PYY and GLP-1. Other examples are SCFAs surviving metabolism by colonic epithelial cells can reach the liver via the hepatic portal vein where propionate stimulates gluconeogenesis, acting as a satiety signal.[131] SCFAs entering the circulation could also interact with FFAR2 and FFAR3 located on adipose tissue, resulting in leptin stimulation. According to a study in mice, acetate, the principal SCFA formed by prebiotic fermentation, can cross the blood-brain barrier and enter the hypothalamus, promoting anorectic signals.[132]

[H1] Translation to the clinic

Box. Overcoming barriers to translation to the clinic

- More high quality, adequately powered randomized, controlled trials that test well-defined probiotic (strain or strain combinations, dose, delivery matrix) and prebiotic interventions on substantive clinical outcomes.
- Better tracking of safety data during the conduct of short and long term clinical trials
- Improved availability of high quality, properly labelled, and effective commercial products[133]
- Application by clinicians of available efficacy data in evidence-based manner. This comprises assessment of totality of data (positive and null) through unbiased systematic review processes for specific probiotic and prebiotic interventions.
- Better understanding of characteristics of host (including diet, baseline microbiota, medications and disease) that improve response to probiotics or prebiotics
- Clinicians need clarification about probiotic and prebiotic products: are they safe, who will benefit – how and to what extent, and can the product labels be trusted

Many clinical gastrointestinal indications could benefit from probiotic and prebiotic interventions. In the case of prebiotics, a link between the clinical benefit to microbiota function should be established. For probiotics, a clinical indication is needed. For both, robust product information is required[133].

There are clinical indications for use of certain probiotic strains supported by robust evidence. In paediatric and/or adult populations, evidence exists for necrotizing enterocolitis (NEC),[134] antibiotic-associated diarrhea (AAD) and *H. pylori* infection[135,136,137], defecation frequency[138,139], infantile colic,[140], mild to moderate ulcerative colitis,[141] irritable bowel syndrome,[142] treatment of acute diarrhea[143] prevention of *C. difficile*-associated diarrhea[144] and neonatal sepsis.[28] A recent meta-analysis provided evidence that probiotic use has the potential to decrease antibiotic utilization in children.[145] Some clinical guidelines have been issued for probiotic use in children.[146,147] Systemic from the gut, evidence exists for reduction of incidence and duration of upper respiratory tract infections.[147,149] No official recommendations have been made for adult uses of probiotics. Additional research clarifying the most effective strains and doses is needed for many clinical targets so far researched.[150,151,152] Although many clinical indications are promising, data are still emerging for endpoints including brain, metabolic, and cardiovascular effects.

Generally, the strength of evidence for prebiotic interventions lags behind those for probiotics. Perhaps the strongest support for prebiotic use comes from prebiotic infant formulae. Such products are now routinely supplemented with mixtures of GOS and fructans[153,154] and this blend of prebiotics in a 9:1 ratio reduced respiratory tract infections to levels found in breast-fed infants.[155,156 101] There is less evidence that prebiotics can reduce infections in adults, although one placebo-controlled, randomized, double blind study of 159 healthy volunteers, showed that GOS could reduce the incidence of diarrhea.[157]

Much of the research focus on prebiotics has been in the realm of functional food (improves well-being through benefit beyond its nutrient content) applications. The one example of a prebiotic food application recognized by European regulatory authorities is on improved bowel function in healthy adults resulting from consumption of 12g of chicory inulin per day.[158,159]

Prebiotic foods designed to increase satiety and reduce energy intake is a promising approach to augment compliance with weight loss diets. Oligofructose-enriched inulin in overweight children has been shown to increase satiety, reduce energy intake as well as BMI and body fat mass over 16 weeks (body weight

decrease of 3.1%, percent body fat decrease of 2.4% compared with children given placebo (increase of 0.5%, increase of 0.05% respectively).[160,161] Oligofructose ingested daily by 29 adults for 12 weeks in a granola bar formulation, reduced by 0·3 (sd 1·2) kg lean mass and waist circumference by -2·2 (sd 3·6) cm, with a concomitant intake and increase satiety in adults over a 12-week intervention.[162] However, not all studies have suggested benefits. One study of 97 overweight or obese children given oligofructose for 12 weeks did not show a statistically significant change in BMI-for-age z-score versus placebo.[163] This study did not measure the effect of the prebiotic on the gut microbiota and its function, which would have provided mechanistic insight to better understand the null study results and enabled better design of future interventions.

The replacement of glycaemic carbohydrates in food products with non-glycaemic carbohydrates to reduce post-prandial glycaemic responses has already received a positive EFSA opinion.[164] Prebiotic carbohydrates might be expected to bring additional benefits in terms of increasing satiety in such a replacement strategy. Promising results were observed from a double-blind, randomized, controlled cross-over trial of 40-42 healthy adults who consumed a yogurt drink containing oligofructose. The intervention improved postprandial glucose responses.[165]

There is now some evidence that the stool microbiota profiles of patients with inflammatory conditions, such as inflammatory bowel disease, differs from those who are heathy,[166] but it is not clear at the present time why. It is unclear whether these differences are caused by the underlying medical condition, are a consequence of the disease pathology, or due to confounding factors such as medications or altered dietary habits. Probiotic or prebiotic interventions hold promise to achieve disease or symptom mitigation through microbiota modulation. An understanding of the microbiome composition and function in the donor and recipient will help us understand the extent to which clinical success depends on these factors.[167] Indeed, some clinical trials have noted the importance of baseline microbiota composition among responders.[168,169,170] Microbiota patterns can be influenced by lifestyle, living conditions, diet, medications and stool consistency, among other transient variables. Advanced age is also thought to be a factor, but one study of Chinese subjects has shown that healthy centenarians have similar microbiota to healthy young people,[171] suggesting that factors other than age are more important drivers of microbiota composition. Furthermore, research methodologies and data management may lead to spurious interpretations of microbiota assessments, which has the potential to mislead.[172] Although clinical benefits have been observed with probiotic and prebiotic

interventions, the onus is on researchers to clarify the role of the microbiome in these successes to optimize short and long-term outcomes.[173,174,175,176,177].

Careful phenotypic and genotypic descriptions of study subjects may also be important to clinical trial success targeting the microbiome. Host genetic studies may help, for example, in the microbiome-mediated disease of IBD, where 163 loci were identified to meet genome-wide significance thresholds.[178] However, since the majority of cases of IBD are not the result of a single host gene defect,[179] complicating the development of clinical interventions based on host genetics. Another complication is that identified genes constitute risk factors, not causal determinants, for a disease, and therefore clinical strategies based on host genomics have not been forthcoming.

IBD comprises two main forms, Crohn's disease and ulcerative colitis. In Crohn's disease, there seems to be distinct molecular subclasses of genomic associations, further complicating development of effective strategies.[180] This may in part explain why probiotic strains have mostly failed to be effective in improving the management of Crohn's disease.[181,182] The reason why mild to moderate ulcerative colitis has been somewhat improved by probiotic intervention[183] but Crohn's is not known. The future success of microbiota manipulation to mitigate serious inflammatory conditions will require an understanding of the interactions between the microbiome and the human genetic risk factors and will necessitate moving beyond microbial genomic sequencing to transcriptomic, metabolomic and proteomic investigations.

The promise of treating or curing disease with microbiota manipulation continues to be explored using probiotic species different from those traditionally employed[184]. Many probiotics in current use are from the genera *Lactobacillus* and *Bifidobacterium*. Although many of them were derived from the faeces or intestinal mucosa of healthy human subjects, researchers today are considering the utility of many newly explored human resident microbes, such as *Akkermansia, Eubacterium, Propionibacterium, Faecalibacterium* and *Roseburia*. This will require going beyond laboratory animal experiments that proliferate in the literature.[185,62]

Faecal microbial transplantation (FMT), which has been a reasonably successful treatment for recurrent *Clostridium difficile* infection,[186,187,188] is being met with mixed success in the treatment of other conditions[189-191]. Although FMT is not a probiotic application since it is not suitably defined microbiologically to meet the probiotic definition,[2] the approach is based on the concept that microbes derived from healthy donor feces can restore proper function to a dysbiotic microbial

ecosystem. It is noteworthy that there have only been a few blinded, randomized controlled trials (RCTs) on FMT for treatment of recurring *C. difficile*, and these have been relatively small studies and we have little information on the long-term changes that such a broad, poorly defined and non-specific treatment might have on individuals. A well-defined reproducible probiotic intervention is more suitable for rigorous research investigation and could be safer long-term than FMT, as suggested by several researchers attempting to assemble a defined consortium of microbes for such purposes [192]. Whether these defined consortia, typically comprising many human commensal microbial species, can reach the same levels of cure as FMT remains to be seen.

The potential impact of gut microbiota manipulation on clinical medicine is promising. However, in the excitement over potential, stakeholders often forget that association does not mean causation. For example, blinded reviews of 34 oesophageal biopsy samples found that these microbiomes could be classified into 2 types. Type I was dominated by the genus *Streptococcus* and was phenotypically normal. But Type II, demonstrating a greater proportion of Gram-negative anaerobes and/or microaerophiles, correlated with oesophagitis and Barrett oesophagus.[150] Like many other microbiome findings, this does not prove causation and there are numerous potential reasons why these associations might exist, including diet, drugs, and lifestyle. One hypothesis might be that administering a safe, select *Streptococcus* could reduce oesophagitis and Barrett's oesophagus, but this has not been tested. Microbiome differences do not necessarily mean that microbiota modification will lead to improved health.

[H1] The future

The gut microbiota might be central to the cause of many disorders and its modulation could hold the key to new effective therapies. So, what are the roles of probiotics and prebiotics? In a general sense, both interventions serve to increase the community of beneficial microorganisms and products of their growth and metabolism in the host. In this context, effects relayed might exert influences systemically, such as in the cardiovascular system, or to the urogenital tract, skin and brain.[193]

The field is poised for conceptual advances. Target microbes will expand beyond the typical *Bifidobacterium* spp. and *Lactobacillus* spp. (as mentioned above)

to include other genera and maybe more yeast species.[194-197] These microorganisms might be new probiotic candidates or further targets for prebiotic utilization.

Improved precision, accuracy, and repeatability of measures of microbial composition, which lead to genuine and not misleading interpretations, are needed in this field.[172] Improved assessments will lead to an expanded range of probiotic and prebiotic products. For example, propionate and butyrate are both considered to be beneficial gut microbial metabolites, but neither is produced by bifidobacteria or lactobacilli.[198,199] Therefore, an opportunity exists to define microbes with metabolic capabilities beyond those afforded by traditional probiotics.

Another development could be anti-adhesive molecules and carbohydrates that attenuate microbial virulence. These factors would be adjuncts to current prebiotic approaches in that they are not selectively utilized substrates.

To have robust proof that gut microbiome alterations can reduce disease incidence or mitigate disease, more well-designed RCTs are needed. By randomly assigning individuals to intervention groups, most biases are reduced and the chances of useful results are improved. Due to the easy availability and relatively cheap cost of high-throughput sequencing technology, microbiota analysis is becoming widespread and differences among disease states increasingly well publicized. The expertise and databases required for metabolomic analysis is also on an upward trend. This will be vital to optimise clinical translation, as a much greater awareness of the functional ecology of the gut is needed together with improved clarity of how this ecosystem influences systemic health. Microbiota and host transcriptomic studies are also important, but they are expensive, time-consuming and require substantial bioinformatic support. Ultimately, the application of probiotic and prebiotic regimens has the potential to improve human health and contribute greatly to how patients are managed and/or disease risk is reduced.

[H1] Conclusions

Although certain commonalities allow us to group substances under the ‘probiotic’ or ‘prebiotic’ umbrellas, benefits to human health are tied to specific products, not the categories *en masse*. To the extent that a clinical outcome is associated with a specific mechanism of action, then it could be hypothesized that a similar strain or prebiotic expressing that mechanism might also be beneficial. However, it is important not to overgeneralize conclusions about specific entities. In general, when an intervention is effective or ineffective, it must be recognized that those results are tied to specific formulations, doses, clinical endpoints and target populations. It is

incumbent upon responsible scientists to consider the totality of available information on specific interventions as a basis of overall conclusions on effectiveness. Furthermore, clinicians should scrutinize both positive and null studies for bias, as only in eliminating bias in research will we move the field toward truth, thereby realising the potential of probiotics and prebiotics.

The body of research suggests that these interventions can not only improve symptomology, but have a meaningful effect on reducing pathology and even saving lives. The prevention of sepsis and NEC in infants provides compelling examples. These findings demonstrate effective translation of human microbiome research. Such clinical impact has changed practice in many healthcare environments. Yet many constituencies have yet to embrace the concept through critically considering the strengths and weaknesses of existing data.

In developing countries, probiotics widely available in developed countries are either not accessible or affordable to most people. However, a program has introduced inexpensive sachets containing a probiotic *L. rhamnosus* (GR-1 or Yoba 2012) plus *S. thermophilus* C106 that allows locals to produce different forms of fermented foods (yoghurt, millet, cereals, juices) that not only influence health but also empower poverty-stricken communities to improve social well-being.[4] With over 260,000 consumers being reached each week in East Africa, the potential is enormous to use these beneficial microbes and local food sources to impact communities (manuscript submitted).

Diseases and poor health often result from the interplay of microbiological and biological ecosystems along with societal issues including pollution, food shortages and poor medical care.[200,201] We encourage more research and translational efforts on probiotics and prebiotics to serve the people of developing countries, who might stand to benefit most from these interventions.

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

Publisher's note

Reviewer information **[Au: placeholder for reviewer information as per our Referee accreditation rules]**

Nature Reviews XXX thanks [Referee#1 name], [Referee#2 name], and other anonymous reviewer(s), for their contribution to the peer review of this work.

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Probiotics and prebiotics in intestinal health and disease: from biology to the clinic

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The gut microbiota has been implicated in a range of diseases with microbiota manipulation suggested as a possible therapeutic approach. This Review describes current understanding of probiotics and prebiotics as a means to manage the microbiota to improve host health, including mechanisms of actions and potential for clinical use.

Figure 1. Probiotic mechanisms of action. Diverse mechanisms are likely to drive probiotic benefits to host health. In some cases, such as production of antimicrobial products and cross-feeding other resident microbes, these mechanisms are driven directly by interactions with the resident microbiota. In other cases, such as direct interaction with immune cells, their effects might be directly via interaction with host cells. Overall, clinical benefits delivered by probiotics could result from the combined action of several mechanisms.

GABA, gamma amino butyric acid.

Figure 2. Identified mechanisms of action of prebiotics

The premise is that prebiotics enter the gut and are selectively utilized. This step increases bacterial growth and functionality of specific genera or species. As a result of either or both of these effects, health benefits can then accrue. Fecal bulking and improved bowel habits occurs due to microbial growth. Immune regulation can be influenced by increased biomass and cell wall components of the bacteria. Metabolic products include organic acids, which lowers intestinal pH and have concomitant effects upon microbial pathogens and mineral absorption. Metabolic products can also influence epithelial integrity and hormonal regulation. Bacteria that respond to prebiotic intake can influence the microbiota composition through elaboration of antimicrobial agents (e.g. peptides) and competitive interactions, possibly reducing infections and bacteria containing LPS.

GLP, glucagon like peptide; IL – interleukin; LPS, lipopolysaccharide; PYY, peptide YY; TGF, transforming growth factor; Th1, type 1 T-helper cell, type 1; Th2, type 2-T helper cell; Tr, regulatory T cell.

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